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18. (Amended). The method of claim 1, further comprising filtering the [supernatant]clarified solution through an ultrafiltration unit comprising a gel layer before contacting the [supernatant]clarified solution with the positively charged ion exchange resin.

REMARKS

Claims 1-20 are pending in the present application. The pending claims after entry of this amendment are presented in Appendix A. In the Office Action, claims 1 and 18 were rejected under 35 U.S.C. § 112, second paragraph, for allegedly being vague and indefinite. Claims 17-19 were provisionally rejected under the doctrine of obviousness-type double patenting over claims 1-62 of the parent application, which issued as US Patent No. 6,011,148. In addition, claims 1-17 were rejected under 35 U.S.C. § 103(a) for allegedly being obvious over Maniatis (*Molecular Cloning: A Laboratory Manual*, 86-91, Cold Spring Harbor Laboratory, 1982), Marquet *et al.* (*BioPharm*, 42-50, May 1997), Pühler *et al.* (US Patent No. 4,621,061), Holmes (US Patent No. 4,830,969) and Wan *et al.* (US Patent No. 5,837,529). Lastly, claims 1-20 were rejected under 35 U.S.C. § 103(a) for allegedly being obvious over Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes and Wan *et al.*, in view of Hrinda *et al.* (US Patent No. 5,300,433), Geiger *et al.* (US Patent No. 5,034,314), van Reis (US Patent No. 5,256,294), Horn *et al.* (US Patent No. 5,707,812), Hubble *et al.* (*Biotechnology Letters*, 7:273-276, 1985), and Rembhotkar *et al.* (*Analytical Biochemistry*, 176:373-374, 1989). Each of the rejections will be addressed in the order in which they were raised.

In the specification

The Examiner has noted that the specification contains the trademarks FRACTOGEL, ULTIPOR, SARTOPURE, MIRACLOTH, CENTRASETTE, VANTAGE A, CENTRAMATE, and MILLIPAK. The specification has now been amended to capitalize each of these trademarks, as requested by the Examiner. Prior to this amendment, generic terminology already accompanied the use of the trademarks in the specification, except for FRACTOGEL on page 19. Support for the addition of the generic term "resin" on page 19 of the specification can be found on page 10, line 31, of the application as originally filed. No new matter is added by this amendment.

In the claims

Claim 1 has been amended to more clearly state Applicants' invention which is directed to the large-scale production of at least about 100 mg of pharmaceutical-grade plasmid DNA. Support for this amendment is found on page 2, lines 12-25, on page 5, lines 27-28, and in the last sentence of the abstract. Thus, no new matter is added by this amendment.

Step (f) of claim 1 has also been amended to indicate more explicitly exactly when neutralization should occur. Support for this amendment is found on page 8, lines 15-27. No new matter is added.

Claim 18 has been amended to more clearly specify that the supernatant solution which is to be filtered through the ultrafiltration unit is the clarified solution produced by the centrifugation step (e). Support for this minor amendment can be found by comparing claim 18 and claim 1 as originally filed. Support is also found on page 11, lines 22-23. No new matter is added.

Rejections under 35 U.S.C. § 112, second paragraph

Claim 1 has been rejected under 35 U.S.C. § 112, second paragraph, as allegedly being vague and indefinite in step (f). The Examiner asserts that step (f) dictates that a precipitation mixture be neutralized after the mixture has been centrifuged, and the claim is therefore unclear.

Step (f) of claim 1, however, merely dictates that either the precipitation mixture or the clarified solution be neutralized. The step does not state that the neutralization of the precipitate mixture should occur after centrifugation of the precipitation mixture. On the contrary, one of ordinary skill in the art will readily recognize that by referring to the "precipitation mixture", Applicants are indicating that any neutralization of the precipitation mixture would have to occur prior to the centrifugation of step (e). As an alternative to this, the clarified solution may instead be neutralized after the centrifugation of step (e). The listing of the neutralization step subsequent to the centrifugation step does not by itself limit the claims to a method where the neutralization step must necessarily follow the centrifugation step. The order of the steps of a method claim is not limited by the order in which the steps are listed in the absence of language to the contrary. In fact, claim 5, to which the Examiner did

not object, is explicitly directed a method of claim 1 where the neutralization step (f) precedes the centrifugation step (e).

Nevertheless, in order to address the Examiner's concerns regarding clarity, Applicants have now amended claim 1 to more clearly state at exactly what stage of the method the neutralization step should occur.

In addition, claim 18 stands rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for lack of sufficient antecedent basis for the phrase "the supernatant". Applicants have now amended claim 18 to replace "supernatant" with the terminology actually used in claim 1 to refer to the supernatant following centrifugation – "clarified solution". Applicants respectfully submit that this amendment addresses the Examiner's concerns.

Obviousness-type double patenting

Applicants note the provisional rejection of claims 17-19 for allegedly being obvious over the allowed claims of the parent application (USSN 08/691,090), now issued as US Patent No. 6,011,148. Applicants will provide an appropriate Terminal Disclaimer, if necessary, when claims 17-19 are indicated as otherwise allowable.

Rejections under 35 U.S.C. § 103(a)

Claims 1-17 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes, and Wan *et al.*.

In this rejection, the Examiner states that Maniatis teaches a basic plasmid purification method including lysing cells with alkali, precipitating the plasmid DNA with potassium acetate, centrifuging the precipitation mixture and neutralizing the clarified solution. Marquet *et al.* and Pühler *et al.* each allegedly teach schemes for "large scale" plasmid purification which include all the steps of the method except the use of static mixers and RNase digestion. Holmes allegedly teaches "large-scale" plasmid purification including the steps of the method except the use of static mixers. Lastly, the Examiner alleges that Wan *et al.* teaches the use of the static mixers in the lysis and precipitation steps.

This rejection is respectfully traversed.

To support a *prima facie* case of obviousness, the references cited by the Examiner must provide motivation to make the claimed invention (MPEP 2143.01) and provide a reasonable expectation of success (MPEP 2143.02). The Examiner suggests that such motivation would be present here because all of the elements of the claims would have been known to one of ordinary skill in the art "to be practiced and combined as suited the situation".

Claims 1-17, as amended, are directed to methods for purifying plasmid DNA suitable for pharmaceutical use from bacterial cells on a large scale. The claimed method produces plasmid DNA of sufficient purity and quantity for pharmaceutical use. Thus, Applicants' methods may be used to produce quantities of at least about 100 mg of pharmaceutical grade plasmid DNA. Applicants' method may be used to purify even 100s of grams of pharmaceutical-grade plasmid DNA. These large quantities of plasmid DNA are isolated by Applicants' methods from many liters of bacterial culture. Typically, at least about 40 liters of bacterial culture would be processed at once (see Examples 1 and 5 of Applicants' specification).

Applicants' method comprises a number of different steps including lysis, clarification, concentration, and chromatography. For each of these steps, a number of different options exist in the art. For instance, lysis techniques known to those of ordinary skill in the art included alkaline lysis, enzymatic lysis, sonication, bead milling and homogenization. Clarification and concentration steps may involve such techniques as ammonium acetate precipitation, polyethylene glycol precipitation, or ultrafiltration. Options for purification of various biomolecules by chromatography include size-exclusion chromatography, reverse-phase chromatography, affinity chromatography and ion exchange chromatography. In addition, a wide variety of techniques exist for the physical manipulations of mixing or treating the solutions at various steps. For instance, the mixing of alkaline lysis may be achieved by such different methods as inversion in a tube, mixing by planery mixer, or mixing by static mixer. A multi-step purification method, therefore, could conceivably include any of a wide number of possible combinations of the various individual techniques.

Although the Examiner has pointed to a number of references which contain disclosure of some of the various possible techniques for each of the steps of Applicants'

invention, the Examiner has not identified how these references suggest the particular combination claimed by Applicants, as opposed to any of the other large number of possible combinations. The Examiner points to no guidance in any of the references which suggests the combination of alkaline lysis in a static mixer with an ion-exchange chromatography step, instead of, for instance, the combination of enzymatic lysis and rotation with a size-exclusion chromatography step. In the absence of specific motivation provided by the cited references, the Examiner's rejection amounts to the application of an improper "obvious to try" standard.

The existence of motivation to combine the cited references is particularly questionable in light of the large scale of Applicants' method. Although references cited by the Examiner may refer to "large-scale" plasmid purification, almost all of the cited references are only instructive as to purification of quantities of media of about 1 liter or less, volumes considered large scale within the laboratory setting, but not within the context of Applicants' invention which is directed to a method useful for the large-scale manufacturing of pharmaceutical-grade plasmid DNA. For instance, although the cited portion of Maniatis is labeled "Large-Scale Isolation of Plasmid DNA", the disclosed protocol teaches the isolation of plasmid DNA from only 500 ml. Cells grown in 500 ml of media will typically result in highly-purified plasmid yields of a few milligrams or less, not plasmid yields of about 100 mg and above as claimed by Applicants. Two other references cited by the Examiner, Holmes and Pühler *et al.*, may also be directed to purifications which are considered large on the laboratory scale, but, again, these references simply do not teach plasmid purification on the large-scale required for the manufacturing of a pharmaceutical. For instance, Holmes and Pühler *et al.* disclose the purification of plasmids from 1 liter and 100 ml of bacterial culture, respectively. These volumes can be contrasted with the volumes of at least 40 liters contemplated by Applicants' invention (see Examples 1 and 5). Applicants' claims are directed to a process of a much larger scale than would, or even could, ordinarily be performed within the standard laboratory setting.

One of ordinary skill in the art would not have assumed that purification techniques which were suitable on the 100 ml scale were necessarily suitable on the 40 liter scale. Teachings of small-scale or even large-scale laboratory preparative methods are not necessarily scalable up to the large scale required for manufacturing a pharmaceutical product

as claimed by Applicants. The purification of large quantities of pharmaceutical-grade plasmid DNA is, in fact, plagued with problems related to the scale of production (for a review of these problems, see Prazeres *et al.*, "Large-scale production of pharmaceutical-grade plasmid DNA for gene therapy: problems and bottlenecks", *TIBTECH*, 17:169-174, 1999). These problems include the difficulties of mixing reagents efficiently on the large scale. Also, the instability of plasmids in solutions derived from cell lysates may be problematic due to the increased time required by large-scale processes. Column capacity and expense may also be issues where chromatography is desired as part of a large-scale preparation procedure. Alkaline lysis provides one example of the types of problems inherent in going from the laboratory scale to the large scale. Although alkaline lysis works very well on the laboratory scale, the extreme viscosity of an alkaline lysis mixture creates considerable problems on the large scale (see column 1, lines 42-64, of Wan *et al.*). Because of these potential problems and others, one of ordinary skill in the art would not necessarily have adapted a technique useful on the laboratory scale to a large-scale method with any reasonable expectation of success.

Furthermore, Applicants' claims are not only drawn to methods providing substantial amounts of purified plasmid (at least about 100 mg), but also plasmid DNA purified to pharmaceutical grade (see claim 1, as amended). Pharmaceutical use requires a much higher level of purity than would ordinarily be required for laboratory use. For example, pharmaceutical-grade DNA must be free of endotoxins, which are not normally a concern in the laboratory. None of the references cited by the Examiner provides guidance as to whether the particular combination of steps claimed by Applicants would have been capable of producing purified plasmid DNA not only in quantities of at least about 100 mg, but also as to whether the produced plasmid DNA would be of sufficient purity to constitute pharmaceutical-grade plasmid DNA. Although Marquet *et al.* addresses the necessity for purity, it provides no guidance on the appropriateness of combining static mixer lysis and precipitation steps with ion exchange columns, and the like. Again, motivation from within the cited references is lacking.

Applicants have identified a superior method for the purification of large quantities of pharmaceutical-grade plasmid DNA, and it is only through impermissible hindsight that Applicants' claimed combination of known techniques, out of the many possible

combinations of techniques, appears obvious. Since no motivation was provided by the cited references of Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes, and Wan *et al.* which would have led one of ordinary skill in the art to combine the references to arrive at the claimed, large-scale process for the purification of pharmaceutical-grade plasmid DNA, the rejection of claims 1-17 as allegedly being obvious over these references is improper.

Furthermore, even if the references could be properly combined, the rejection of claims 6-11 is still improper because not all elements of the claims are provided by the cited combination of references. It is well settled that a *prima facie* case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, must provide all of the elements of the claimed invention. Yet, the Examiner has pointed to no prior art which teaches the specific static mixer limitations introduced by dependent claims 6-11. (Claims 6 and 9 are directed to specific flow velocities and diameters. Claims 7 and 11 are limited to 24-element static mixers. Claims 8 and 10 are limited to laminar flow static mixers.) As shown in Applicants' specification (pages 21-23, Examples 2-4; page 7, lines 14-20), the limitations of claims 6-11 are the result of experimental optimization of static mixer conditions for the large-scale production of purified plasmid DNA for pharmaceutical use. The claimed conditions appear to be notable different from those in the cited art. Wan *et al.* only discloses a 32-element static mixer (column 4, lines 7-10), not the 2-element static mixer of claims 7 and 11. The Examiner also has not alleged that the use of such specific static mixer parameters would have been obvious to one of ordinary skill in the art. Thus, the Examiner has failed to establish a *prima facie* case of obviousness for claims 6-11.

Further indication that the cited combination of references does not render Applicants' invention obvious can be found in the evidence of a long-felt need for a large-scale process for the purification of large quantities of pharmaceutical-grade plasmid DNA. A long-felt, but unsatisfied need, for an invention while the needed elements have long been available is a secondary consideration to be taken into account in the determination of obviousness. *See Graham v. John Deere*, 383 US 1, 17-18 (1966).

The long-felt need for large-scale production of pharmaceutical-grade plasmid DNA is evident from Wan *et al.* (column 1, lines 7-67; filed in 1996, published in 1997 as WO 97/23601), and the long-felt need for high levels of purity is evident from Marquet *et al.*

(p. 42, 1st and 2nd columns; published in 1997). Yet, the review article Prazeres *et al.*, published years later in 1999, makes it abundantly clear that the problems of large-scale purification of pharmaceutical-grade plasmid DNA had still not been satisfactorily solved two to three years after the writing of Wan *et al.* and Marquet *et al.*. Prazeres *et al.* write, "There are several problems and bottlenecks associated with the design and operation of large-scale processes for the production of pharmaceutical-grade plasmid DNA for gene therapy." (abstract). They also write, "Solutions for these and other problems or alternative processing technologies are highly desirable, and will undoubtedly have an impact on the economics, efficacy and safety of non-viral approaches to gene therapy." (page 173). Thus, despite the fact that the teachings of Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes, and Wan *et al.* had been available to those of ordinary skill in the art for years, there is no evidence that the teachings of these references had been combined as suggested by the Examiner to produce a method suitable for the large-scale purification of pharmaceutical-grade plasmid DNA.

In addition, Applicants wish to note that although the Examiner has asserted that Marquet *et al.*, Holmes, and Pühler *et al.* all disclose ion exchange chromatography, Applicants only find mention of the use of ion exchange chromatography for the purification of plasmid DNA in one of the references, Pühler *et al.* (a laboratory-scale disclosure). Holmes appears to disclose laboratory-scale plasmid purification by size-exclusion chromatography (column 6, lines 53-58; column 4, lines 9-11) and cesium chloride (column 5, line 21) but not by ion exchange chromatography. Marquet *et al.*, mentions the use of anion exchange columns, but only for the purpose of *characterizing*, not preparing or purifying, plasmid DNA (see page 46, 2nd column, first whole paragraph). The method taught by Marquet *et al.* is taught only as an analytical tool, not as a preparative tool on even the laboratory scale. If anything, Marquet *et al.* teaches away from the adaptation of the ion exchange column for large-scale preparations of DNA by indicating that the recommended ion exchange column has a very low sample capacity (page 46, 2nd column, end of first whole paragraph), a huge problem for scalability.

In light of the foregoing amendments and remarks, Applicants respectfully request that the rejection of claims 1-17 under 35 U.S.C. § 103(a) as allegedly being obvious over Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes, and Wan *et al.* be withdrawn.

Claims 1-20 stand rejected under 35 U.S.C. § 103(a) for allegedly being obvious over Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes, and Wan *et al.*, in view of Hrinda *et al.*, Geiger *et al.*, van Reis, Horn *et al.*, Hubble *et al.*, and Rembhotkar *et al.*.

In this rejection, the Examiner states that it would have been obvious to combine the method to concentrate, fractionate, purify and desalt a protein using polarization gel layer in a ultrafiltration device as allegedly taught by Hrinda *et al.* with the methods allegedly taught by Geiger *et al.*, van Reis, Horn *et al.*, Hubble *et al.*, and Rembhotkar *et al.* because they all allegedly teach the advantageous use of polarization gel layers in ultrafiltration devices to concentrate, fractionate, purify and desalt compositions comprising DNA's.

This rejection is respectfully traversed.

As discussed above, to support a *prima facie* case of obviousness, the references cited by the Examiner must provide motivation to make the claimed invention and provide a reasonable expectation of success. As also discussed above, the references Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes and Wan *et al.* fail to provide any motivation for combining all the steps disclosed in these references to arrive at the claimed large-scale process for purifying pharmaceutical-grade plasmid DNA. The citation of five additional references for a total combination of no less than *eleven* disparate, cited references, does not change this. The Examiner still points to nothing in the prior art which suggests the claimed *combination* of steps. For example, the Examiner still points to no prior art which suggests the desirability of combining alkaline lysis in a static mixer with ultrafiltration.

The Examiner does claim that Horn *et al.* teaches the combination of ultrafiltration and ion exchange chromatography. However, Horn *et al.* actually teaches gel filtration chromatography, not ultrafiltration. Applicants' specification defines ultrafiltration as a technique to separate particles by filtration through a membrane having pore sizes ranging from about 0.001 to about 0.05 μm (page 5, lines 9-17; page 11, line 22, to page 15, line 9). Horn *et al.* refers only to the use of a column packed with sephacryl resin, not a semipermeable membrane. Thus, *Horn et al.* fails to suggest Applicants' combination of ultrafiltration and ion exchange chromatography.

Furthermore, one of ordinary skill in the art would not have been motivated to combine the cited references to produce the claimed method for the purification of plasmid DNA, because those references cited by the Examiner which do teach ultrafiltration, do not teach ultrafiltration conditions suitable for the purification of plasmids. Hrinda *et al.* and Hubble *et al.* only teach the use of ultrafiltration for the purification of proteins, not nucleic acids. The Examiner correctly noted that Hrinda *et al.*'s disclosure is limited to proteins. However, Hubble *et al.* only appears to discuss the ultrafiltration of proteins (page 274, Table 1). Also, although the Examiner asserts that Rembhotkar *et al.* teaches the fractionation and concentration of DNA by tangential flow ultrafiltration, a close reading of the reference indicates that it only teaches the purification of whole bacteriophage *particles*, not phage *DNA*. As can be seen on page 374 of Rembhotkar *et al.*, the step involving isolation of the DNA from the bacteriophage (step "(iii)") occurs only after the ultrafiltration step (step "(ii)"). Rembhotkar *et al.*, therefore, teaches nothing relating to ultrafiltration of nucleic acids themselves. Similarly, the van Reis reference teaches the use of ultrafiltration for the purification of proteins at great length. Although the reference does suggest that ultrafiltration could be used for other species of suitable size, including DNA, van Reis does not teach conditions which would be suitable for plasmid DNA purification. For instance, column 8, lines 54-59, of van Reis teaches the use of high velocity, high cross-flow conditions which are conditions that would likely shear DNA.

One of ordinary skill in the art would not have assumed that a technique which was useful for purifying protein would necessarily also be suitable for purifying plasmid DNA. Proteins and plasmid DNA differ in their physical properties in a number of critical ways that could easily affect the suitability of a particular purification scheme. For instance, proteins are generally globular; plasmids are not. Plasmids are subject to shearing; proteins are generally not. Proteins are often largely hydrophobic; plasmid DNA is highly charged and hydrophilic. One simply cannot assume that a purification technique which works for proteins would also work for nucleic acids of plasmid size, and vice versa. For example, phenol extraction at a certain pH works well as a step in the purification of DNA. However, one of ordinary skill in the art would not assume that such a technique as phenol extraction would be suitable as a step in the isolation of a protein from a cell lysate. Accordingly, it cannot be said that one of

ordinary skill in the art would have been particularly motivated to use ultrafiltration to purify plasmids simply because a reference indicated that ultrafiltration was useful for protein purification.

Also, although Geiger *et al.* teaches the use of ultrafiltration for a purification of nucleic acids, it does not teach the purification of plasmid DNA from cell contaminants as Applicants do. Rather, Geiger *et al.* teaches the purification of single-stranded probe polynucleotides away from the hybridized, double-stranded polynucleotides. The ultrafiltration conditions taught are directed towards a very specific goal, the removal of excess labeled probe (typically consisting of short DNA sequences) after attempted hybridization to such species as chromosomal DNA or rRNA. Geiger *et al.* would have failed to guide one of ordinary skill in the art to adapt the disclosed ultrafiltration technique to the separation of plasmid DNA from cell contaminants.

Furthermore, although the Examiner asserts that one of ordinary skill in the art would have been motivated to combine the cited references to purify plasmid DNA to pharmaceutical grade because many of the references "taught the advantageous use of (polarization) gel layers in ultrafiltration devices", Applicants strongly disagree. Applicants contend that none of the cited references teaches the favorability of the gel layer for purification of plasmid DNA from cell lysates and therefore none of the references provides the requisite motivation claimed by the Examiner.

Hrinda *et al.*, van Reis, Horn *et al.*, Hubble *et al.*, and Rembhotkar *et al.* all teach that the formation of a gel layer on the membrane during ultrafiltration is undesirable, not advantageous. Example 4 of column 15 of Hrinda *et al.* reveals that Hrinda *et al.* considered the formation of a polarization gel layer to be problematic. In Part A of Example 4 of Hrinda *et al.*, the extreme low yield of ultrafiltration was blamed on the gel layer. Part B of Example 4 shows an attempt to move to a membrane with a higher molecular weight cut-off to circumvent the problem, but still, only moderate results were achieved. Likewise, a close reading of the van Reis reference reveals that van Reis considers formation of the gel layer during ultrafiltration to be undesirable (column 2, lines 1-20, lines 43-47, lines 60-64; column 4, lines 32-44). For instance, van Reis states that one object of the invention involves ultrafiltration processes which minimize concentration polarization (column 4, lines 60-64). Similarly,

Hubble *et al.* are highly concerned about the "fouling" of the membrane associated with formation of a polarization gel layer (page 273, Introduction). The entire reference is devoted to the exploration of a new possible type of hydrophilic membrane which the authors hope will be less subject to gel layer formation when used in ultrafiltration. Any reference which refers to a phenomenon as "fouling" and seeks to diminish the occurrence of the phenomenon cannot be said to teach the advantages of the phenomenon or to have motivated those of ordinary skill in the art to pursue that phenomenon. Horn *et al.* do not teach ultrafiltration and therefore do not teach the desirability of a gel layer on the ultrafiltration membrane. Hubble *et al.* teach how to avoid or diminish the formation of a gel layer, not the advantages of using a gel layer. Similarly, Rembhotkar *et al.* promote the use of tangential flow ultrafiltration while saying that precisely this technique helps keep concentration polarization from forming a gel layer on the membrane surface. Again, such a statement cannot logically be construed as promoting the desirability of a gel layer.

In fact, Geiger *et al.*, appears to be the only cited reference which teaches the favorability of a gel layer in certain situations. However, Geiger *et al.* only teaches the favorability of a gel layer for the specific purpose of separating hybridized polynucleotides from unhybridized probe, two molecules of similar structural configuration. There is no suggestion in Geiger *et al.* that a gel layer might be particularly advantageous for the separation of an entity like plasmid DNA from proteins, cell membrane debris, carbohydrates, and the like. One of ordinary skill in the art would not have assumed that the surprisingly desirable result achieved by Geiger *et al.* in the removal of short probe polynucleotides would have been generally applicable to the separation of plasmid DNA from many other types of contaminants. Again, a technique taught for removal of one type of entity will not necessarily be useful in the removal of other types of contaminants.

Therefore, since no motivation can be found in the cited references of Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes, Wan *et al.*, Hrinda *et al.*, Geiger *et al.*, van Reis, Horn *et al.*, Hubble *et al.*, and Rembhotkar *et al.* which would have led one of ordinary skill in the art to combine the teachings of the references to arrive at the claimed, large-scale process for the purification of pharmaceutical-grade plasmid DNA, the rejection of claims 1-20 as allegedly being obvious over these references is improper.

As a final note, with regard to Applicants' claim 20, Applicants wish to point out that the references Hrinda *et al.*, Geiger *et al.*, van Reis, and Hubble *et al.* fail to disclose ultrafiltration comprising tangential flow ultrafiltration with an open channel device (as required by claim 20). For instance, Hrinda *et al.*, teaches a stirred cell system, not tangential flow ultrafiltration (column 14, lines 45-48). Geiger *et al.* and Hubble *et al.* teach "dead end" ultrafiltration, not tangential flow ultrafiltration, and therefore cannot be teaching the use of an open channel (Geiger *et al.*: column 7, lines 14-29; *Hubble et al.*: page 274, lines 10-11). The van Reis reference does teach tangential flow ultrafiltration, but only appears to teach the use of screened channels, not open channels. Thus, none of these cited references provides the requisite ultrafiltration elements of claim 20.

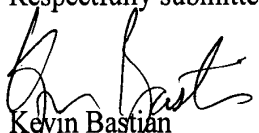
In light of the foregoing amendments and remarks, Applicants respectfully request that the rejection of claims 1-20 under 35 U.S.C. § 103(a) for allegedly being obvious over Maniatis, Marquet *et al.*, Pühler *et al.*, Holmes and Wan *et al.*, in view of Hrinda *et al.*, Geiger *et al.*, van Reis, Horn *et al.*, Hubble *et al.*, and Rembhotkar *et al.* be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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